

# **Contents**

---

<i>Preface .....</i>	(v)
<i>Acknowledgement.....</i>	(vii)

## **CHAPTER 1**

### **Chromatography**

1.1 Introduction.....	1
1.2 Various Types of Chromatography.....	1
1.3 Techniques based on Solute-stationary Phase Interaction .....	1
1.3.1 Adsorption Chromatography .....	1
1.3.2 Partition Chromatography .....	2
1.3.3 Ion Exchange Chromatography .....	2
1.4 On the basis of Chromatographic Bed Shape.....	2
1.4.1 Column Chromatography .....	2
1.4.2 Paper Chromatography .....	2
1.4.3 Thin layer Chromatography.....	3

## **CHAPTER 2**

### **Paper Chromatography**

2.1 Introduction.....	4
2.2 Principle of Paper Chromatography .....	4
2.3 Uses and Application of Paper Chromatography .....	4
2.4 Types or Modes of Paper Chromatography .....	5
2.5 Procedure in Paper Chromatography .....	7
2.6 Quantitative Analysis.....	10
2.7 Qualitative Analysis.....	10
2.8 Applications of Paper Chromatography.....	11

## **CHAPTER 3**

### **Thin layer Chromatography**

3.1 Introduction .....	13
3.2 Principle of Thin Layer Chromatography .....	13
3.3 Procedure of Thin layer Chromatography.....	14
3.4 Procedure .....	15
3.4.1 Plate Preparation.....	15
3.4.2 Capillary Spotters .....	15
3.4.3 Spotting the Plate.....	15
3.4.4 Location of Spots.....	16
3.4.5 Development Solvents.....	16
3.4.6 Mobile Phase .....	17
3.4.7 Developing a Plate.....	17
3.4.8 Visualization.....	18
3.4.9 Analysis .....	18
3.5 Applications of Thin layer Chromatography .....	19

## **CHAPTER 4**

### **Gas Liquid Chromatography**

4.1 Introduction .....	22
4.2 Principle .....	22
4.2.1 Theoretical Plates (Plate Theory) .....	23
4.2.2 HETP - Height Equivalent to a Theoretical Plate) .	23
4.2.3 Efficiency (No. of Theoretical Plate).....	24
4.3 Instrumentation .....	24
4.4 Applications of GLC .....	31

## **CHAPTER 5**

### **Ion Exchange Chromatography (or Iron Chromatography)**

5.1 Introduction .....	32
5.2 Ion Exchange Chromatography Principle .....	32
5.3 Types of Ion Exchange Resins .....	34
5.4 Factors Affecting Ion Exchange Separations .....	36
5.5 Applications of Ion Exchange Chromatography .....	37

## **CHAPTER 6**

### **Column Chromatography**

6.1	Introduction.....	38
6.2	Principle .....	38
6.3	Procedure in Column Chromatography.....	39
6.4	Factors Affecting Column Efficiency .....	44
6.5	Applications of Column Chromatography .....	44
6.6	Advantages of Column Chromatography.....	45

## **CHAPTER 7**

### **Electrophoresis**

7.1	Introduction.....	46
7.2	Classification of Electrophoresis.....	47
7.3	Paper Electrophoresis.....	48
7.3.1	Introduction .....	48
7.3.2	Definition.....	48
7.3.3	Principle of Paper Electrophoresis.....	49
7.3.4	Components of Paper Electrophoresis.....	49
7.3.5	Types of Paper Electrophoresis .....	50
7.3.6	Factors Affecting Migration of Ions .....	52
7.3.7	Applications.....	53
7.3.8	Advantages .....	53
7.3.9	Disadvantages.....	54
7.4	Gel Electrophoresis .....	54
7.4.1	Introduction .....	54
7.4.2	Principle.....	54
7.4.3	Types of Gel Electrophoresis.....	54
7.4.3.1	Agarose Gel Electrophoresis.....	54
7.4.3.2	SDS-Page .....	59
7.4.3.3	Pulse Field Gel Electrophoresis .....	64
7.4.3.4	Two-Dimensional Electrophoresis.....	66
7.5	Cellulose Acetate Electrophoresis.....	68

7.6	Moving Boundary Electrophoresis .....	70
7.7	Zone Electrophoresis.....	72
7.7.1	Types of Zone Electrophoresis .....	72

## **CHAPTER 8A**

### **Radioimmuno Assay (RIA)**

8A.1	Introduction.....	74
8A.2	Components of Radioimmuno Assay.....	75
8A.3	Structure of Antibodies .....	76
8A.4	Principle of Radioimmunoassay .....	76
8A.5	Immune Reactions.....	76
8A.6	Mathematical Expression of Principle of RIA .....	78
8A.7	Reagents used in Radioimmuno Assay .....	78
8A.8	Steps Followed in Radioimmuno Assay .....	78
8A.9	Detailed Procedure in Radioimmuno Assay .....	79
8A.10	Advantages of Radioimmuno Assay.....	79
8A.11	Disadvantages of Radioimmuno Assay .....	80
8A.12	Uses of Radioimmuno Assay .....	80
8A.13	Limitations of Radioimmuno Assay .....	80

## **CHAPTER 8B**

### **ELISA**

8B.1	Introduction.....	81
8B.2	Procedure .....	81
8B.3	Types of ELISA .....	82
8B.3.1	Direct ELISA .....	82
8B.3.2	Indirect ELISA .....	83
8B.3.3	Sandwich ELISA .....	84
8B.3.4	Competition/Inhibition ELISA .....	85
8B.4	ELISA Detection Options - Direct & Indirect .....	87
8B.4.1	Direct ELISA Detection .....	87
8B.4.2	Indirect ELISA Detection.....	88
8B.5	Bioluminescence Assay .....	88

## **CHAPTER 9**

### **X-ray Crystallography**

9.1 History.....	91
9.2 X-ray Diffraction.....	91
9.3 Production of X-rays.....	92
9.3.1 Properties .....	93
9.4 Methods of X-ray Diffraction .....	94
9.5 Bragg's Law .....	95
9.6 Source of X- ray .....	96
9.6.1 Coolidge Tube .....	96
9.6.2 Synchrotron Radiation .....	97
9.7 Radioisotopes .....	98
9.7.1 Atomic Number and Mass Number.....	98
9.7.2 Radioisotope .....	99
9.8 Rotating Crystal Technique.....	99
9.8.1 The Laue Method.....	99
9.8.2 Experimental Procedure for Powder Diffraction ..	103
9.9 Pharmaceutical Applications of X-Ray Diffraction .....	104

## **CHAPTER 10**

### **Introduction to Spectroscopy**

10.1 Introduction.....	107
10.2 Electromagnetic Radiation.....	107
10.3 Electromagnetic Spectrum .....	107
10.4 Definition of Spectroscopy .....	108
10.5 Types of Spectroscopy .....	109
10.6 Ultraviolet Spectroscopy.....	110
10.7 Theory .....	110
10.8 Principle (Lambert Beer Law) .....	113
10.8.1 Deviation of Beer-Lambert Law.....	115
10.8.2 Real Limitation and Deviation of Beer-Lambert Law.....	115

10.9	Instrumentation .....	117
10.10	Types of UV Spectrophotometer .....	123
	10.10.1 UV Spectrophotometer Single Beam System.....	123
	10.10.2 UV Spectrophotometer Double Beam UV System.....	124
10.11	Advantage of Double Beam Spectrophotometer.....	124
10.12	Effects in UV Transitions.....	125
10.13	Chromophore .....	125
	10.13.1 Effect of Conjugation .....	126
10.14	Pharmaceutical Applications.....	127
10.15	Woodward- Fieser Rules for Calculating Absorption Maxima .....	129
	10.15.1 Woodward- Fieser rules for calculating $\lambda_{\max}$ in conjugated dienes, trienes and polyenes....	129
	10.15.2 Woodward- Fieser rules conjugated dienes, triples and polyenes.....	130
	10.15.3 Woodward-Fieser rules for $\alpha$ , $\beta$ -unsaturated carbonyl compounds.....	131

## **CHAPTER 11**

### **Infra-Red Spectroscopy**

11.1	Introduction .....	132
11.2	The Origin of IR Spectra.....	133
11.3	Theory .....	133
11.4	Calculation of Vibrational Frequencies .....	137
	11.4.1 Polyatomic Vibrational Spectra.....	138
	11.4.2 Factors Influencing Vibrational Frequencies.....	138
11.5	Instrumentation .....	141
	11.5.1 Single Monochromator Infrared Spectrophotometers .....	141
11.6	Applications of IR Spectroscopy .....	145

## **CHAPTER 12**

### **Fluorimetry**

12.1	Introduction.....	147
12.2	Types of Luminescence.....	147
12.3	Principle of Fluorescence Spectroscopy .....	148
12.4	Instrumentation .....	149
12.5	Factors Affecting Fluorescence.....	151
12.6	Effect of Concentration on Fluorescence Intensity .....	152
12.7	Advantages of Fluorescence Spectroscopy .....	152
12.8	Disadvantages of Fluorimetry Spectroscopy.....	153
12.9	Applications of Fluorimetry .....	153
12.9.1	Applications in Inorganic Chemistry.....	153
12.9.2	Applications in Organic Chemistry .....	153
12.9.3	Special Fluorometric Applications .....	154

## **CHAPTER 13**

### **Flame photometry**

13.1	Introduction.....	156
13.2	Principle of Flame Photometry .....	156
13.3	Instrumentation .....	157
13.4	Flame Photometry Procedure.....	164
13.5	Scheibe Lomakin Equation .....	165
13.6	Application of Flame Photometer .....	165
13.7	Advantages of Flame Photometer .....	166
13.8	Disadvantages of Flame Photometer.....	166

## **CHAPTER 14**

### **Atomic Absorption Spectroscopy**

14.1	Introduction.....	167
14.2	Principle .....	167
14.3	Instrumentation .....	168

14.4	Working of AAS .....	172
14.5	Applications of Atomic Absorption Spectroscopy.....	172

## CHAPTER 15

### Nepheloturbidimetry

15.1	Introduction.....	173
15.2	Principle and Theory of Nephelometry and Turbidimetry ....	173
15.3	Theory .....	174
15.4	Working Conditions of Nephelometry and Turbidimetry .....	174
15.5	Instrumentation of Nephelometry and Turbidimetry .....	175
15.5.1	Experimental Techniques of Nephelometry and Turbidimetry .....	176
15.5.2	Calculation.....	176
15.6	Applications of Turbidimetry and Nephelometry .....	176

## CHAPTER 16

### NMR Spectroscopy

16.1	Introduction.....	178
16.2	Definition of NMR.....	179
16.3	Principle of NMR .....	179
16.3.1	The Spinning Molecules.....	179
16.3.2	Magnetic and Non-Magnetic Nuclei .....	179
16.4	Theory of NMR.....	180
16.4.1	Energy Transition .....	181
16.5	Nuclear Magnetic Resonance Spectrometers Instrumentation.....	182
16.5.1	Experimental Technique.....	183
16.5.2	Instrument.....	184
16.6	Chemical Shift.....	184
16.6.1	Units.....	185
16.6.2	Measurement of Chemical Shift .....	185

16.6.3	Causes of Chemical Shift.....	185
16.6.4	Shielding and Deshielding Effects.....	186
16.7	Factors Affecting Chemical Shift.....	187
16.7.1	Spin-spin Coupling.....	190
16.7.2	Peak Splitting in NMR Spectroscopy .....	190
16.7.3	Pascal's Triangle.....	191
16.7.4	Factors Influencing Peak Split in NMR Spectrum Due to Spin-spin Coupling .....	192
16.7.5	Determination of Singlets and Doublets.....	192
16.7.6	Unit of Coupling Constant.....	193
16.7.7	Different types of couplings and their effects on the coupling constant .....	193
16.7.8	Complex Splitting Patterns on NMR Spectrum....	194
16.8	Interpretation of NMR Spectrum .....	194
16.8.1	Spectrum of-Isomers-dimethyl Ether and Ethanol .....	195
16.9	Applications of NMR Spectroscopy .....	196
16.10	Nuclear Magnetic Double Resonance (NIVIDR) .....	197
16.11	Electron Paramagnetic Resonance (EPR) .....	197

## **CHAPTER 17**

### **Mass Spectroscopy**

17.1	Introduction.....	198
17.2	Principle .....	198
17.3	Instrumentation .....	199
17.4	Types of Ion Produced .....	205
17.5	Mass Spectrum.....	207
17.6	Fragmentation Patterns.....	208
17.7	McLafferty Rearrangements.....	211
17.8	Application of Mass Spectroscopy.....	212